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Contributions to species of *Xylariales* in China—2. *Rosellinia pervariabilis* and *R. tetrastigmae* spp. nov., and a new record of *R. caudata*

Xin Xie[1,2], Lili Liu[3,4], Xu Zhang[3,4], Qingde Long[3,4], Xiangchun Shen[3,4], Saranyaphat Boonmee[5], Jichuan Kang[1*A], Qirui Li[1,3,4*B]

[1] *Engineering and Research Center for Southwest Bio-Pharmaceutical Resources of National Education Ministry of China &*
[2] *Department of Plant Pathology, College of Agriculture Guizhou University, Guiyang, Guizhou 550025, China*
[3] *State Key Laboratory of Functions and Applications of Medicinal Plants, Guizhou Medical University, Guiyang 550004, China*
[4] *The Key Lab of Optimal Utilization of Natural Medicine Resources, School of Pharmaceutical Sciences, Guizhou Medical University, University Town, Guian New District, Guizhou 550025, China*
[5] *Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand*

[*] Correspondence to: [A] *jckang@gzu.edu.cn* [B] *lqrnd2008@163.com*

Abstract—Three species of *Rosellinia* collected from China are reported based on morphological and molecular characteristics. *Rosellinia pervariabilis* is proposed as a new species, differentiated by its narrow ascospores (19.5–24.5 × 4–5 μm) without a germ slit. *Rosellinia tetrastigmae* is also proposed as new and differentiated by its small stromata. *Rosellinia caudata* is reported as a new record from the Chinese mainland. Detailed morphological descriptions and illustrations are provided, and many new DNA sequences are cited.

Key words—*Ascomycota*, pyrenomycetous fungi, *Xylariaceae*, taxonomy

Introduction

This is the second in a planned series of papers on *Xylariales* from China (Long & al. 2019). *Rosellinia,* introduced by De Notaris (1844), a large genus

in terms of species number in the *Xylariaceae* Tul. & C. Tul. (Wijayawardene & al. 2017). Despite the recent resurrection of *Hypoxylaceae* DC, *Rosellinia* remains assigned to *Xylariaceae* (Wendt & al. 2018, Daranagama & al. 2018, Wijayawardene & al. 2018). *Rosellinia* is a species rich genus, especially in tropical and subtropical areas (Petrini 1992, 2003, 2013a). In 2013, 142 *Rosellinia* species were accepted based on the morphological studies of numerous specimens (including many type specimens) from different parts of the world (Petrini 2013a). Currently, 41 species of *Rosellinia* have been reported from China (Teng 1963, Tai 1979, Ju & Rogers 1990, 1999; Yuan & Zhao 1993, Lu & al. 2000, Liu & al. 2010, Petrini 2013a, b; Li & Guo 2015, 2016, 2018; Li & al. 2015, Su & al. 2016). The taxonomic concept of *Rosellinia* and the terminology applied in this paper follow Petrini (2013a).

During our investigation of *Xylariales* in China, two new species and one new record of *Rosellinia* were found from Guizhou province. In addition, newly obtained DNA sequence data are reported in this paper.

Materials & methods

Collection & isolation

Fresh materials collected in southwestern China were brought to the laboratory in paper bags. Macroscopic characters were examined under an Olympus SZ61 stereomicroscope and photographed with a fitted Canon 700D digital camera (Senanayake & al. 2015). Materials were mounted in water and Melzer's reagent for anatomical examination; asci, ascospores, and ascus apical rings were photographed using a Nikon digital camera fitted to a light microscope. More than 30 ascospores, 20 asci, and 20 apical rings were measured for each specimen. Pure colonies were obtained by single-spore isolation following Chomnunti & al. (2014), and successful cultures were preserved in 2 ml screw cap centrifuge tubes with 10% glycerol at –20 °C and with sterile water at 4 °C. Herbarium materials were deposited at the herbarium of Guizhou Medical University, Guizhou, China (GMB) and the Herbarium of Cryptogams, Kunming Institute of Botany, Academia Sinica, Yunnan, China (KUN-HKAS). Cultures were deposited at Guizhou Medical University Culture Collection (GMBC).

DNA extraction, PCR amplification, sequencing

Colonies were transferred to 2% potato-dextrose agar (PDA) medium and incubated at 25 °C until the hyphae filled the plate. Fresh mycelia were scraped off the medium with a clean scalpel blade. Total genomic DNA was extracted from fresh mycelia using a Biomiga GD2416 Fungus Genomic DNA Extraction Kit (Su & al. 2016). DNA products were preserved at –20 °C.

ITS rDNA regions were amplified with primer pairs ITS4 and ITS5; α-actin gene (ACT) sequence fragments were amplified with primer pairs ACT512F and ACT783R

Table 1. Taxa of *Rosellinia* and related genera used in the molecular analyses.

Taxon	Culture number	GenBank acc. no.		Reference
		α-actin	β-tubulin	
Amphilogia gyrosa	YMJ 91123101	EF025600	EF025615	Hsieh & al. 2010
Annulohypoxylon nitens	YMJ 91022108	AY951772	AY951663	Hsieh & al. 2005
A. squamulosum	YMJ 90081905	AY951774	AY951665	Hsieh & al. 2005
Astrocystis bambusae	89021904 (HAST)	GQ449239	GQ495942	Hsieh & al. 2010
A. mirabilis	94070803 (HAST)	GQ449238	GQ495941	Hsieh & al. 2010
A. sublimbata	89032207 (HAST)	GQ449236	GQ495940	Hsieh & al. 2010
Biscogniauxia anceps	YMJ 123 [**T**]	AY951783	AY951671	Hsieh & al. 2005
B. arima	YMJ 122	AY951784	AY951672	Hsieh & al. 2005
B. capnodes	YMJ 138	AY951787	AY951675	Hsieh & al. 2005
B. mediterranea	YMJ 147	AY951796	AY951684	Hsieh & al. 2005
B. simplicior	YMJ 136	AY951798	AY951686	Hsieh & al. 2005
Collodiscula leigongshanensis	GZUH0107 [**T**]	—	KR002587	Li & al. 2015
C. fangjingshanensis	GZUH0109 [**T**]	—	KR002589	Li & al. 2015
Daldinia caldariorum	YMJ 263	AY951802	AY951690	Hsieh & al. 2005
D. childiae	CBS 122881	KU684039	KU684129	U'Ren & al. 2016
*Durotheca comeden***S**	BCC25152	GQ160480	GQ160488	Læssøe & al. 2013
D. depressa	BCC28073	GQ160484	GQ160492	Læssøe & al. 2013
Hypoxylon rubiginosum	YMJ 24	AY951862	AY951751	Hsieh & al. 2005
H. shearii var. *minor*	YMJ 29	AY951864	AY951753	Hsieh & al. 2005
Jackrogersella cohaerens	YMJ 310	AY951766	AY951655	Hsieh & al. 2005
Kretzschmaria clavus	YMJ 114	EF025596	EF025611	Hsieh & al. 2010
K. guyanensis	89062903 (HAST)	GQ408901	GQ478214	Hsieh & al. 2010
K. lucidula	YMJ 112	EF025595	EF025610	Hsieh & al. 2010
K. megalospora	YMJ 229	EF025594	EF025609	Hsieh & al. 2010
Nemania abortiva	467 (BISH) [**T**]	GQ374123	GQ470219	Hsieh & al. 2010
N. illita	YMJ 236 [**T**]	EF025593	EF025608	Hsieh & al. 2010
N. primolutea	YMJ 91102001[**T**]	EF025592	EF025607	Hsieh & al. 2010
N. serpens	235 (HAST)	GQ389695	GQ470223	Hsieh & al. 2010
Podosordaria muli	167 (WSP) [**T**]	GQ455450	GQ844839	Hsieh & al. 2010
Poronia pileiformis	88113001 (WSP)[**T**]	GQ455449	GQ502720	Hsieh & al. 2010
Rosellinia aquila	MUCL 51703	—	KX271253	Wendt & al.2018
R. buxi	99 (JDR)	GQ398228	GQ470228	Hsieh & al. 2010
R. caudata	**GMBC0145**	**MH771031**	**MH778499**	**This study**
	GMBC0207	**MH771029**	**MH778497**	**This study**
	GMBC0208	**MH771030**	**MH778498**	**This study**
R. convexa	GZUCC13005 [**T**]	KF885726	KF885725	Su & al. 2016
R. corticium	MUCL 51693	—	KX271254	Wendt & al. 2018
R. lamprostoma	YMJ 89112602	EF025589	EF025604	Hsieh & al. 2010

R. necatrix	YMJ 89062904	EF025588	EF025603	Hsieh & al. 2010
R. pervariabilis	**GMBC0092 [T]**	**MH771027**	**MH778495**	**This study**
R. sancta-cruciana	90072903 (HAST)	GQ389699	GQ470227	Hsieh & al. 2010
R. tetrastigmae	**GMBC0030 [T]**	**MH771028**	**MH778496**	**This study**
Stilbohypoxylon elaeidicola	YMJ 173	EF025601	EF025616	Hsieh & al. 2010
S. quisquiliarum	YMJ 89091608	EF025591	EF025606	Hsieh & al. 2010
Theissenia pyrenocrata	TC11480	GQ247716	GQ247717	Læssøe & al. 2013
Whalleya microplaca	YMJ 91111215	EF025599	EF025614	Hsieh & al. 2010
Xylaria acuminatilongissima	623 (HAST) [T]	GQ853046	GQ502711	Hsieh & al. 2010
X. brunneovinosa	720 (HAST) [T]	GQ853041	GQ502706	Hsieh & al. 2010

Note: New sequences in **bold**; ex-type sequences designated by '[T]'

(Carbone & Kohn 1999); the β-tubulin gene (BT) was amplified using primer pairs T11 and T1 (Tanaka & al. 2009; Hsieh & al. 2010). Amplification conditions followed Li & al. (2015) and Su & al. (2016). After DNA was amplified using the polymerase chain reaction (PCR), the PCR products were sent to Sino Geno Max in Beijing for DNA sequencing. All nucleotide sequences obtained were uploaded into GenBank, and the accession numbers of DNA sequences used in this paper are listed in Table 1.

Sequence alignment and phylogenetic analyses

Preliminary BLAST searches of ITS rDNA sequences placed our sequences close to those of *Rosellinia* spp. Sequences used for constructing phylogenetic tree in this paper were chosen according to the latest authoritatively published papers and the results of preliminary BLAST searches (Su & al. 2016, Wendt & al. 2018, Daranagama & al. 2018).

Sequences were downloaded and aligned using BioEdit (Hall 1999). The combined dataset was concatenated from individual ACT and BT alignments, with alignments checked and improved manually where appropriate and uploaded in TreeBASE (https://treebase.org/treebase-web/home.html). We used an online tool (http://sing.ei.uvigo.es/ALTER/) to convert files for RAxML and MrBayes (Ronquist & al. 2012). The NEXUS file was modified with MrModeltest v. 2.2 (Nylander 2004).

Randomized accelerated maximum likelihood (RAxML) was conducted using RAxML online (https://www.phylo.org/portal2/). Parameters for RAxML followed Phookamsak & al. (2015). Bayesian analysis was performed with MrBayes v.3.1.2 (Ronquist & al. 2012) via a uniform [GTR+I+G] model (lset nst = 6, rates = invgamma, Prsetstatefreqpr = dirichlet, 1, 1, 1, 1) inferred by MrModeltest 2.3. The phylograms were visualized in Figtree 1.4.3 and polished in Adobe Photoshop version CS6 (Fig. 1).

Results

Three species of *Rosellinia*, two new species and a new record for the Chinese mainland, were recorded.

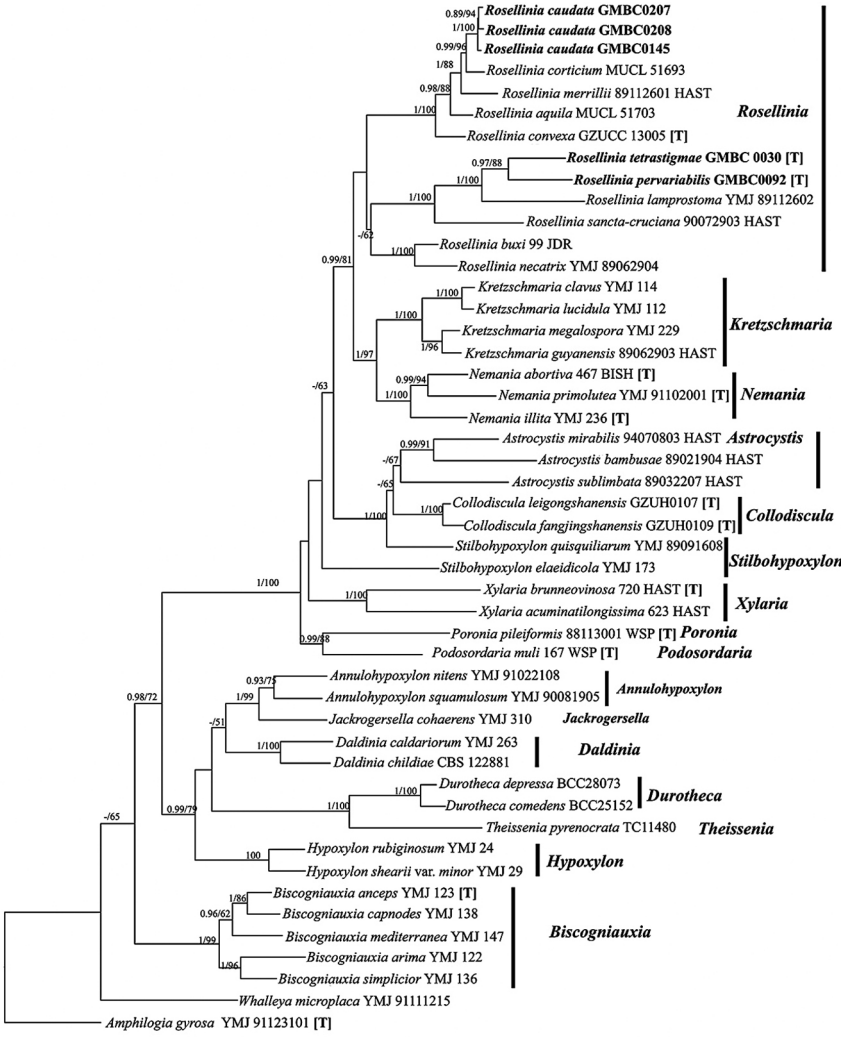

Fig. 1. RAxML tree for *Rosellinia* and related genera based on a combined dataset of β-tubulin and α-actin gene segment sequences. Bayesian posterior probabilities >0.95 and bootstrap support values for maximum likelihood (ML) higher than >50% are marked above the nodes; an en-dash ("–") indicates a value <0.95 (PP) or <50% (BS). Strain numbers are noted after the species names. Our new sequences are shown in bold; and ex-type sequences are annotated with '[T]'. The tree is rooted via the outgroup *Amphilogia gyrosa* YMJ 91123101.

Phylogenetic analyses

Phylogenetic trees were generated with the RAxML and MrBayes methods by using combined datasets of BT and ACT sequences. Forty-seven fungal strains representing 15 different genera were analysed. After manual adjustment, the alignments consisted of 1130 character positions in the BT alignment and 334 in the ACT alignment, of which 92 characters were variable, 715 constant, and 657 parsimony informative. Two different molecular markers were used, and two tree reconstruction methods were applied.

The result of the phylogenetic analysis is shown in Fig. 1 with the final ML optimization likelihood value of –21722.880899. Our isolates fell within genus *Rosellinia*. Our strains formed separate branches on the phylogenetic tree obtained via most parsimonious analysis of the ITS rRNA gene dataset (supplementary material). *Rosellinia tetrastigmae* and *R. pervariabilis* showed a close relationship with *R. lamprostoma* Syd. & P. Syd., with *R. pervariabilis* and *R. tetrastigmae* supported as independent species. Our *R. caudata* three specimens clustered together with *R. corticium* with high bootstrap values (0.99/96).

Taxonomy

Rosellinia pervariabilis Q.R. Li & J.C. Kang, **sp. nov.** Fig. 2

MycoBank MB 827512

Differs from *Rosellinia culmicola* by its smaller ascospores and the absence of a germ slit.

Type—China, Guizhou Province: Qiannan Buyei & Miao Autonomous Prefecture, Maolan National Nature Reserve, on the stem of *Bambusa pervariabilis* McClure, VII 2016, Li 16029 (**Holotype**, GMB 0092; **isotype**, HKAS 101452; ex-type living culture, GMBC 0092).

Etymology—In reference to the host, *Bambusa pervariabilis*.

Saprobic on stem of *B. pervariabilis*. Sexual morph—Subiculum composed of finely interwoven hypha, brown to reddish brown, persistent. Stromata scattered to gregarious, solitary, superficial, subglobose to conical, dark, 0.3–0.5 mm diam., 0.4–0.8 mm high, each containing a single perithecium. Ostioles finely papillate. Ectostroma 20–40 μm thick, black, carbonaceous. Entostroma confined to base, black. Perithecia globose to subglobose. Paraphyses hyaline, septate. Asci 90.5–138 × 9.5–15 μm, 8-spored, cylindrical, unitunicate, short-pedicellate, with a J+ (staining blue in iodine), long barrel-shaped apical ring, 7.5–9 μm high, 3–4.5 μm diam. Ascospores 19.5–24.5 × 4–5 μm, overlapping uniseriate, botuliform, ends

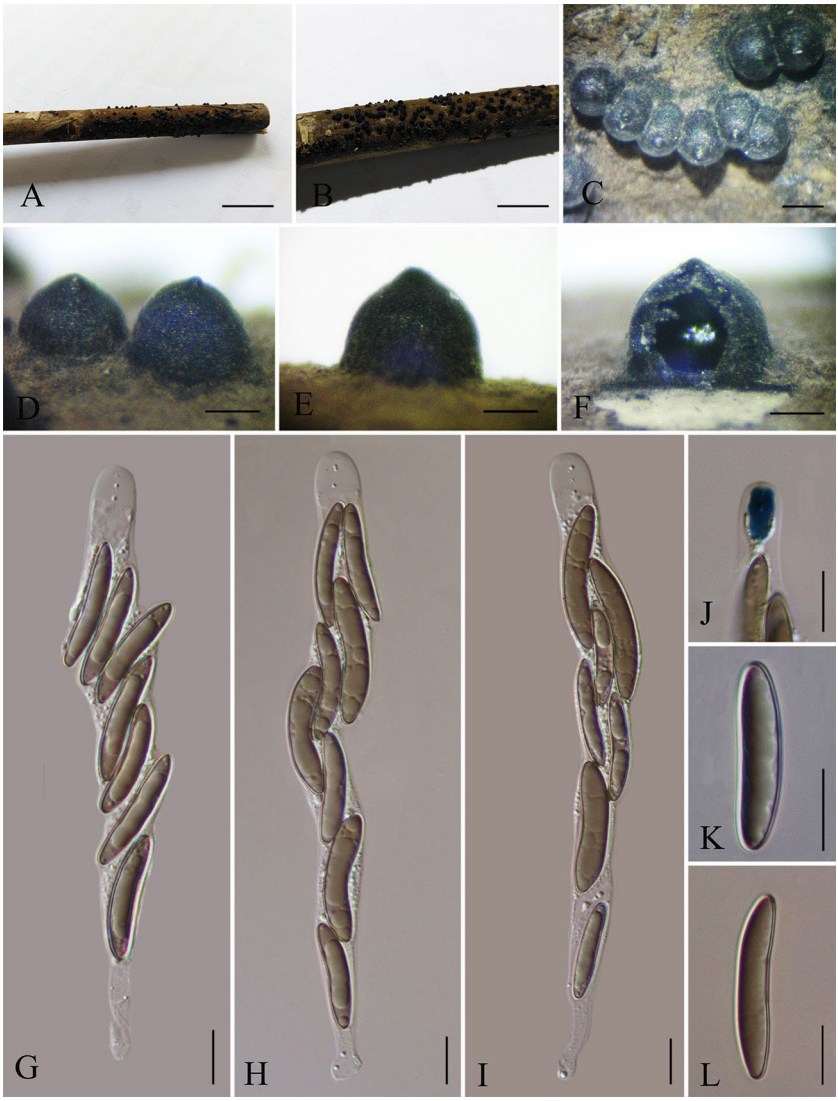

Fig. 2. *Rosellinia pervariabilis* (holotype, GMB 0092). A–E. Stromata on the host. F. Section of stroma. G–I. Asci. J. Long barrel-shaped J+ apical ring. K, L. Ascospores. Scale bars: A, B = 0.2 cm; C, D = 500 μm; E, F = 200 μm; G–L = 10 μm.

rounded, brown to yellowish brown, unicellular, smooth, lacking sheath and germ slit. Asexual morph: unknown.

Comments: *Rosellinia pervariabilis* is diagnosed by its uniquely shaped ascospores and lack of a germ slit. Although there are many *Rosellinia* species producing ascospores within the 15–25 µm range (e.g., *R. abscondita* Rehm, *R. helvetica* L.E. Petrini & al., *R. hyalospora* Theiss), their ascospores have a much lower (≤4) length : width ratio (Petrini 1992, 2003, 2013a). *Rosellinia culmicola* has larger ascospores (24.2±1.5 × 5.9±0.6 µm) and a 6–7 µm long germ slit; moreover, it has a stromatal ring (Petrini 2013a).

Rosellinia tetrastigmae Q.R. Li & J.C. Kang, **sp. nov.** Fig. 3

MycoBank MB 828157

Differs from *Rosellinia megalosperma* by its smaller stromata; and from *R. procera* by its smaller stromata and its ascospores having a germ slit.

Type—China, Guizhou Province: Qiannan Buyei & Miao Autonomous Prefecture, Maolan National Nature Reserve, on the stem of *Tetrastigma sp.* (*Vitaceae*), VII 2016, Li 16012 (**Holotype**, GMB 0030; **isotype**, HKAS 101457; ex-type living culture, GMBC 0030).

Etymology—In reference to the host genus, *Tetrastigma*.

Saprobic on the twigs of dicot wood. Sexual morph—Subiculum composed of finely interwoven hypha, black, persistent. Stromata scattered or gregarious, solitary, superficial, cupulate to oblate sphere, 0.4–0.6 mm diam., 0.3–0.5 mm high, each containing a single perithecium, embedded in the subiculum slightly. Ostioles finely papillate or integrated. Ectostroma 25–50 µm thick, black, carbonaceous. Entostroma black, confined to stroma base. Perithecia subglobose, not collapsed. Paraphyses hyaline, septate. Asci 185–240 × 32–45 µm, 8-spored, cylindrical, overlapping biseriate, unitunicate, short-pedicellate, with a J+, barrel-shaped apical ring, 16–22.5 µm high, 8.5–11.5 µm diam. Ascospores 72.5–111.5 × 12.5–19.5 µm, asymmetrically ellipsoidal with broadly round ends, yellowish brown to dark brown, smooth, surrounded at each end by slimy caps, with a straight germ slit extending the length of the spore on the flat side. Asexual morph: unknown.

Additional material examined—**China, Guizhou Province, Qiannan Buyei & Miao Autonomous Prefecture,** Maolan National Nature Reserve, on the twigs of unknown plant, VIII 2017, Li 17103 (GMB 0212).

Comments: *Rosellinia tetrastigmae* resembles *R. procera* Syd. & P. Syd. and *R. megalosperma* Syd. & P. Syd. San Martín & Roger (1995). Although Ju & Roger (1999) regarded *R. procera* and *R. megalosperma* as synonyms, Petrini (2013a) treated them as independent species based on the presence of ascospore germ slits in *R. megalosperma* and their absence in *R. procera*. *Rosellinia*

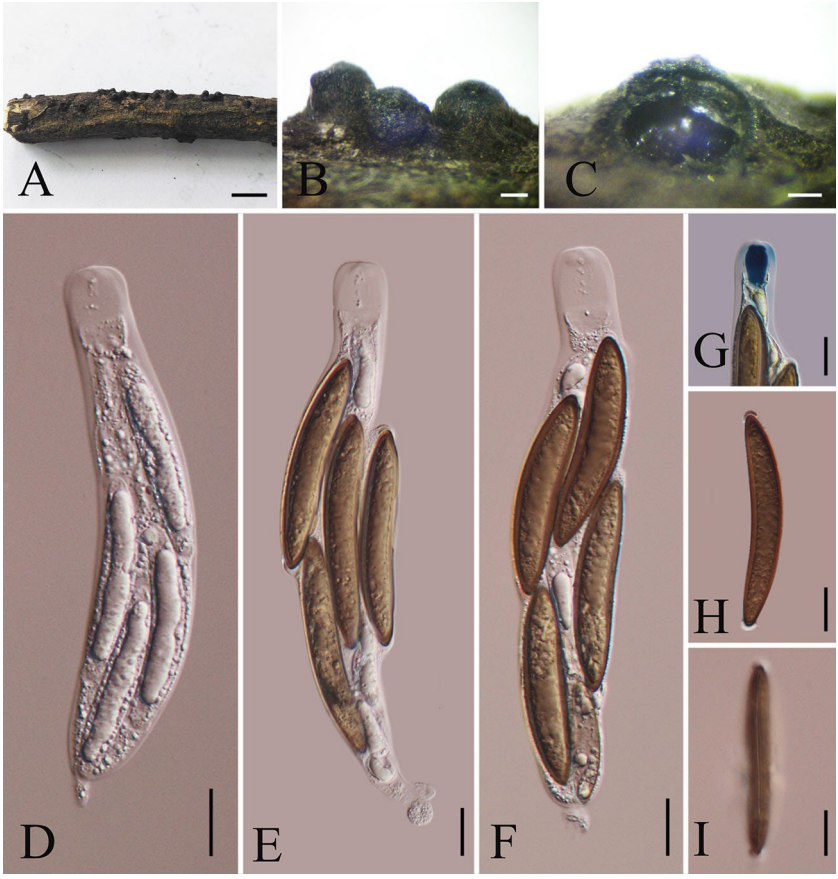

Fig. 3. *Rosellinia tetrastigmae* (holotype, GMB 0030). A, B. Stromata on the host. C. Section of stroma. D–F. Asci. G. Barrel-shaped J+ apical ring. H, I. Ascospores. Scale bars: A = 0.5 cm; B = 200 μm; C= 100 μm; D–I = 20 μm.

tetrastigmae differs from both by its smaller stromata (*R. megalosperma* —600–1000 μm high, 750–1100 μm wide; *R. procera*—825–975 μm high, 550–875 μm wide; Petrini 2013a).

Rosellinia caudata Petch, Ann. Roy. Bot. Gard. (Peradeniya) 10: 135 (1926) Fig. 4

Saprobic on the twigs of dicot wood, forming on the host surface. Sexual morph—Subiculum composed of finely interwoven hypha, well developed, brown to red brown, persistent. Stromata scattered or densely gregarious,

superficial, subglobose to cupulate, dark brown, 0.8–1.2 mm diam., 0.7–1 mm high, containing single perithecia. Ostioles finely papillate. Ectostroma 50–110 µm thick, black, carbonaceous. Entostroma confined to base, black. Perithecia subglobose to cupulate. Paraphyses hyaline, septate. Asci 165–245 × 7.5–15.5 µm, 8-spored, cylindrical, unitunicate, short-pedicellate, with a J+ (staining blue in iodine), long barrel-shaped apical ring, 6–13.5 µm high, 5.5–7.5 µm diam. Ascospores 21.5–30.5 × 7–11 µm, slightly overlapping uniseriate, asymmetrically ellipsoidal with broadly rounded ends, cellular appendage at one end in mature ascospores, brown to dark brown, unicellular, smooth, with a slimy sheath, on convex side with straight germ slit as long as spore. Asexual morph: unknown.

Material examined—**CHINA, Guizhou Province, Qiannan Buyei & Miao Autonomous Prefecture**, Maolan National Nature Reserve, on twigs of unknown plant, VII 2016, Li 16102 (GMB 0145; living culture, GMBC 0145); **Guiyang,** Guizhou Medical University Campus, on twigs of dicotyledonous wood, IV 2017, Li 17115 (GMB 0207; living culture, GMBC 0207); Huaxi Yingbinguan Hotel Park, on twigs of dicotyledonous wood, VII 2017, Li 17126 (GMB 0208; living culture, GMBC 0208).

Comments: Our strains were identified as *Rosellinia caudata,* which are morphologically similar to *R. corticium* (Schwein.) Sacc., *R. aquila* (Fr.) Ces. & De Not., and *R. merrilli* Syd. & P. Syd. (Petrini 2013a). *Rosellinia caudata* also shows a close affiliation with *R. corticium*, *R. merrilli*, and *R. aquila* in the phylogenetic tree (Fig. 1); however, *R. corticium* differs by its larger stromata, *R. aquila* differs by its smaller ascospores and larger stromata, and *R. merrilli* differs by its larger ascospores with a cellular appendage at each end (Petrini 2013a). Petrini (2013a) reported a specimen of *R. caudata* from Taiwan, but we believe that our specimens represent a new record for the Chinese mainland. We also report the first BT and ACT sequences for *R. caudata*.

Discussion

Morphological characteristics are widely used to distinguish between similar species of *Rosellinia*. Stroma shape, presence of subiculum, texture and color, apical ring, ascospore shape and dimensions are important diagnostic characters for *Rosellinia* (Petrini 2005, 2013a). *Rosellinia* species with similarly sized ascospores clustered together clearly in the ITS-based phylogeny by Su & al. (2015). Our analyses show a similar trend (Fig. 1). However, out of more than 142 taxa very few sequences have been uploaded to GenBank, suggesting that *Rosellinia* is still not yet well studied phylogenetically. Much herbarium material is in poor condition and cannot

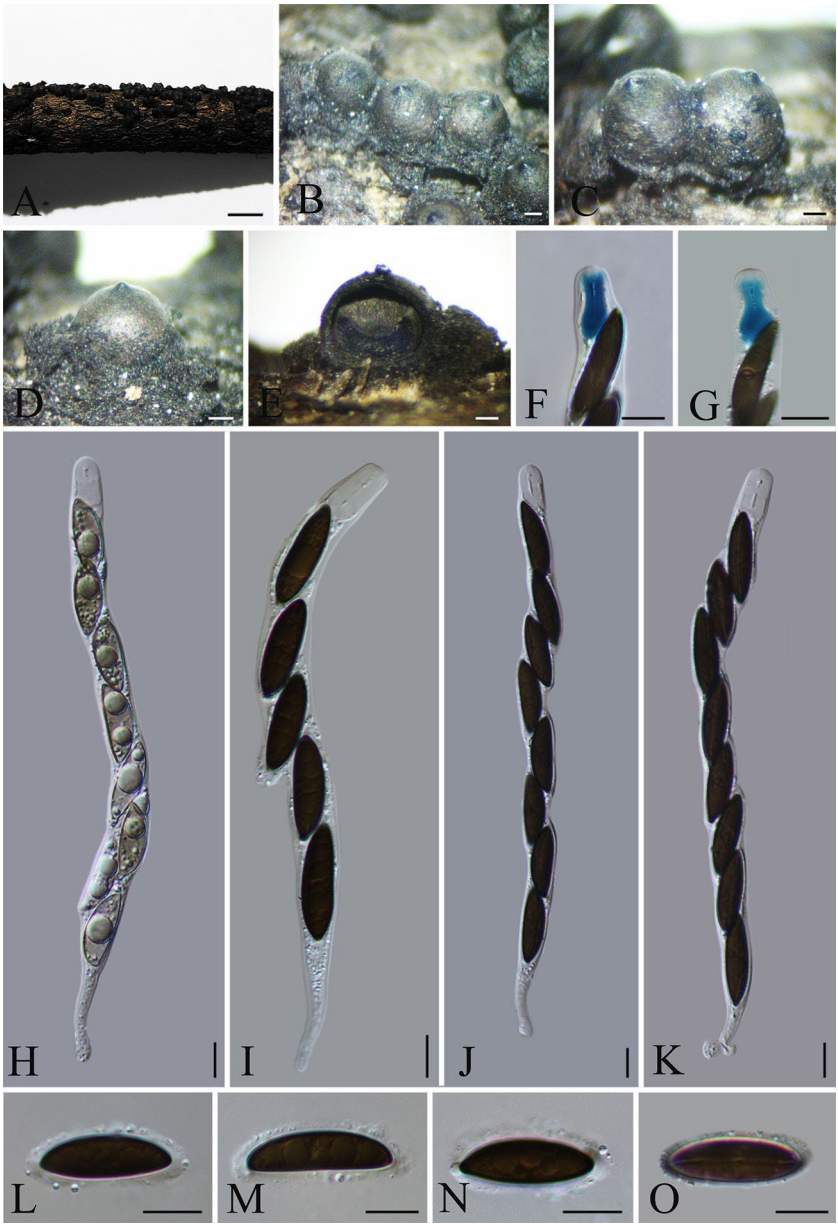

Fig. 4. *Rosellinia caudata* (GMB 0145). A–D. Stromata on the host. E. Section of stroma. F, G Barrel-shaped J+ apical ring. H–K. Asci. L–O. Ascospores. Scale bars: A = 0.5 cm; B–E = 200 μm; F–O = 10 μm.

be used for sequencing. This paper provides sequences from new *Rosellinia* collections, which should prove beneficial to systematic molecular taxonomy of the *Rosellinia*.

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